Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	163549	heinz.in. or girke.in. or scheffler. in. or costa.in. or schmidt.in. or reski.in. or zahringer.in. or BASF$. as.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/09/21 12:39
L2	3439	desaturase	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:39
L3	0	".delta.6" near3 l2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:39
L4	119	delta-6 near3 l2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:39
L5	139	physcomitrella near2 patens	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:40
L6	119	l4 and l4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:40
L7	53	l1 and l2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:40
L8	4	l7 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:40
L9	20	l2 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:40
L10	4	l9 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:41
L11	15601	transgenic near2 plant	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:41
L12	2161	l11 and l2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:41

L13	16	l12 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:41
L14	301	"6" near2 l2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:42
L15	9	l14 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:42
L16	1	l15 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:42
L17	28114	unsaturated near2 "fatty acids"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:43
L18	61	l17 and l4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:43
L19	15	l17 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:43
L20	503	l17 and l11	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:43
L21	145	l17 and l14	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:43
L22	3	l18 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:44
L23	6	l19 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:44
L25	20	l20 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:44

L26	8	l21 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:44
L27	7	l21 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:46
L28	15239	(|435/68.1|435/70.1|435/71.1|435/783|435/252.3|435/419|435/254|.ccls.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:47
L29	137	l28 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:47
L30	26	l29 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:47
L31	0	l30 and l14	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:47
L32	0	l30 and l4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:47
L33	24	l28 and l4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:47
L34	3	l33 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:48
L35	51	l28 and l14	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:48
L36	6	l35 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/09/21 12:48

	Document ID	Title
1	US 20050160496 A1	Production of cereal grain with reduced starch granule size and uses thereof
2	US 20050086713 A1	Transgenic plants containing altered levels of steroid compounds
3	US 20040175722 A1	Methods and compositions for reducing screening in oligonucleotide-directe-d nucleic acid sequence alteration
4	US 20040111763 A1	Novel elongase gene and method for producing multiple-unsaturated fatty acids
5	US 20040053379 A1	Method of producing polyunsaturated fatty acids, novel biosynthesis genes, and novel plant expression constructs
6	US 20040049805 A1	Method for the expression of biosynthetic genes in plant seeds using multiple expression constructs
7	US 20040019927 A1	Polynucleotides and polypeptides in plants
8	US 20040016024 A1	Temporal seed promoters for expressing genes in plants
9	US 20030236208 A1	Targeted chromosomal genomic alterations in plants using modified single stranded oligonucleotides

	Document ID	Title
10	US 20030229918 A1	Seed specific USP promoters for expressing genes in plants
11	US 20030167525 A1	Desaturase genes and uses thereof
12	US 20030157144 A1	Desaturase genes and uses thereof
13	US 20030150008 A1	Transgenic plants containing altered levels of steroid compounds
14	US 20030135877 A1	Sugar and lipid metabolism regulators in plants
15	US 20020069426 A1	Methyl-D-erythritol phosphate pathway genes
16	US 20010051335 A1	POLYNUCLEOTIDES AND POLYPEPTIDES DERIVED FROM CORN TASSEL
17	US 6841717 B2	Methyl-D-erythritol phosphate pathway genes
18	US 6822142 B2	Transgenic plants containing altered levels of steroid compounds
19	US 6635451 B2	Desaturase genes and uses thereof
20	WO 200102591 A	Production of unsaturated fatty acids, useful e.g. in nutrition, cosmetics or pharmaceuticals, in organisms transformed with Physcomitrella patens delta-6-desaturase nucleic acid

	Document ID	Title
1	US 20050086713 A1	Transgenic plants containing altered levels of steroid compounds
2	US 20030167525 A1	Desaturase genes and uses thereof
3	US 20030157144 A1	Desaturase genes and uses thereof
4	US 20030150008 A1	Transgenic plants containing altered levels of steroid compounds
5	US 6822142 B2	Transgenic plants containing altered levels of steroid compounds
6	US 6635451 B2	Desaturase genes and uses thereof
7	WO 200102591 A	Production of unsaturated fatty acids, useful e.g. in nutrition, cosmetics or pharmaceuticals, in organisms transformed with Physcomitrella patens delta-6-desaturase nucleic acid

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  FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 12:53:27 ON 21 SEP 2005
L1       167780 S HEINZ?/AU OR GIRKE?/AU OR SCHEFFLER?/AU OR COSTA?/AU OR SCHMI
L2         7624 S DESATURASE
L3         7803 S UNSATURATED (2W) "FATTY ACID"
L4         1411 S "6" (2W) DESATURASE
L5         1632 S (PHYSCOMITRELLA (2W) PATENS) OR PATENS
L6            0 S TRANSGENIC NEAR2 PLANT
L7         4243 S TRANSGENIC (2W) PLANT
L8          114 S L1 AND L2
L9           11 S L8 AND L5
L10           5 DUP REM L9 (6 DUPLICATES REMOVED)
L11         365 S L2 AND L3
L12          51 S L11 AND L4
L13           1 S L12 AND L5
L14          12 S L2 AND L5
L15           6 DUP REM L14 (6 DUPLICATES REMOVED)
L16           8 S L7 AND L3
L17           8 DUP REM L16 (0 DUPLICATES REMOVED)
L18           6 S L4 AND L5
L19           4 DUP REM L18 (2 DUPLICATES REMOVED)
L20         365 S L2 AND L3
L21          11 S L20 AND REVIEW
L22          10 DUP REM L21 (1 DUPLICATE REMOVED)
L23       10824 S HETEROLOGOUS (3W) EXPRESSION
L24          98 S L23 AND L2
L25           4 S L24 AND L7
L26           4 DUP REM L25 (0 DUPLICATES REMOVED)
L27           0 S L24 AND "IN ANIMAL"
L28           0 S L27 AND "CELL CULTURE"
L29           0 S L24 AND "CELL CULTURE"
L30          24 S L24 AND "ELEGANS"
L31           9 DUP REM L30 (15 DUPLICATES REMOVED)
L32          59 S L24 AND CEREVI?
L33          27 DUP REM L32 (32 DUPLICATES REMOVED)
L34          13 S L33 NOT PY>=2001

=>
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L13 ANSWER 1 OF 1 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 2000230423 EMBASE

TITLE: A bifunctional Δ6-fatty acyl acetylenase/**desaturase** from the moss Ceratodon purpureus: A new member of the cytochrome b5 superfamily.

AUTHOR: Sperling P.; Lee M.; Girke T.; Zahringer U.; Styrune S.; Heinz E.

CORPORATE SOURCE: E. Heinz, Institut fur Allgemeine Botanik, Ohnhorststr. 18, D-22609 Hamburg, Germany. eheinz@botanik.uni-hamburg.de

SOURCE: European Journal of Biochemistry, (2000) Vol. 267, No. 12, pp. 3801-3811.
Refs: 36
ISSN: 0014-2956 CODEN: EJBCAI

COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20000720
Last Updated on STN: 20000720

AB Many plant genes have been cloned that encode regioselective desaturases catalyzing the formation of cis-unsaturated fatty acids. However, very few genes have been cloned that encode enzymes catalyzing the formation of the functional groups found in unusual fatty acids (e.g. hydroxy, epoxy or acetylenic fatty acids). Here, we describe the characterization of an acetylenase from the moss Ceratodon purpureus with a regioselectivity differing from the previously described Δ12-acetylenase. The gene encoding this protein, together with a Δ **6-desaturase**, was cloned by a PCR-based approach with primers derived from conserved regions in Δ5-, Δ6-fatty- acid desaturases and Δ8-sphingolipid desaturases. The proteins that are encoded by the two cloned cDNAs are likely to consist of a N-terminal extension of unknown function, a cytochrome b5-domain, and a C-terminal domain that is similar to acyl lipid desaturases with characteristic histidine boxes. The proteins were highly homologous in sequence to the Δ **6- desaturase** from the moss **Physcomitrella patens**. When these two cDNAs were expressed in Saccharomyces cerevisiae, both transgenic yeast cultures desaturated Δ9-unsaturated C16- and C18-fatty acids by inserting an additional Δ6-cis-double bond. One of these transgenic yeast clones was also able to introduce a Δ6-triple bond into γ-linolenic and stearidonic acid. This resulted in the formation of 9,12,15-(Z,Z,Z)-octadecatrien-6-ynoic acid, the main fatty acid found in C. pupureus. These results demonstrate that the Δ6-acetylenase from C. pupureus is a bifunctional enzyme, which can introduce a Δ6-cis-double bond into 9,12,(15)-C18-polyenoic acids as well as converting a Δ6-cis-double bond to a Δ6-triple bond.

=>

L15 ANSWER 1 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005341104 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15769252
TITLE: In vivo characterization of the first acyl-CoA Delta6-**desaturase** from a member of the plant kingdom, the microalga Ostreococcus tauri.
AUTHOR: Domergue Frederic; Abbadi Amine; Zahringer Ulrich; Moreau Herve; Heinz Ernst
CORPORATE SOURCE: Biozentrum Klein Flottbek, Universitat Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany.. fredomergue@viola.fr
SOURCE: Biochemical journal, (2005 Jul 15) 389 (Pt 2) 483-90. Journal code: 2984726R. ISSN: 1470-8728.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
OTHER SOURCE: GENBANK-AY746357
ENTRY DATE: Entered STN: 20050706
Last Updated on STN: 20050706

AB Genomic DNA of Ostreococcus tauri, a fully sequenced marine unicellular alga from the phytoplankton, was used to amplify a gene coding for a typical front-end **desaturase** involved in polyunsaturated fatty acid biosynthesis. Heterologous expression in Saccharomyces cerevisiae revealed very high desaturation activity with Delta6-regioselectivity. Short-time kinetic experiments showed that the **desaturase** product was detected in the acyl-CoA pool 5 min after addition of the exogenous substrate to the yeast medium and long before its appearance in the total fatty acids. When this **desaturase** was co-expressed with the acyl-CoA Delta6-elongase from **Physcomitrella patens** and the lipid-linked Delta5-**desaturase** from Phaeodactylum tricornutum, high proportions of arachidonic or eicosapentaenoic acid were obtained, because nearly all of the Delta6-desaturated products were elongated. Furthermore, the product/educt ratios calculated in each glycerolipid for the Delta6-**desaturase** or for the acyl-CoA Delta6-elongase were in about the same range, whereas this ratio showed a very uneven profile in the case of the lipid-linked Delta5-**desaturase**. Finally, a sequence-based comparison of all the functionally characterized Delta6-desaturases showed that this enzyme was not related to any previously described sequence. Altogether, our data suggest that this **desaturase** from O. tauri is an acyl-CoA Delta6-**desaturase**, the first one cloned from a photosynthetically active organism.

L15 ANSWER 2 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:219501 BIOSIS
DOCUMENT NUMBER: PREV200510002914
TITLE: Metabolic engineering of new fatty acids in plants.
AUTHOR(S): Singh, Surinder P.; Zhou, Xue-Rong; Liu, Qing; Stymne, Sten; Green, Allan G. [Reprint Author]
CORPORATE SOURCE: CSIRO Plant Ind, POB 1600, Canberra, ACT 2601, Australia Allan.Green@csiro.au
SOURCE: Current Opinion in Plant Biology, (APR 2005) Vol. 8, No. 2, pp. 197-203.
ISSN: 1369-5266.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Jun 2005
Last Updated on STN: 10 Jun 2005

AB Metabolic engineering of plants to express high levels of new fatty acids that are of nutritional and industrial importance has proven to be highly challenging. Significant advances have been made recently, however, particularly in the development of the first plant oils to contain long-chain polyunsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. Methods of increasing the accumulation of Delta 12-modified fatty acids synthesized by

transgenically expressed FAD2-like enzymes have also been investigated. Biochemical analyses of plants that express these introduced fatty-acid metabolic pathways have highlighted the central importance of ensuring the removal of novel fatty acids from their site of synthesis on phosphatidylcholine to enable their further modification, exclusion from membrane lipids and accumulation in seed triacylglycerols.

L15 ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:17247 BIOSIS
DOCUMENT NUMBER: PREV200500017046
TITLE: Novel fatty acid elongases and their use for the reconstitution of docosahexaenoic acid biosynthesis.
AUTHOR(S): Meyer, Astrid; Kirsch, Helene; Domergue, Frederic; Abbadi, Amine; Sperling, Petra; Bauer, Joerg; Cirpus, Petra; Zank, Thorsten K.; Moreau, Herve; Roscoe, Thomas J.; Zahringer, Ulrich; Heinz, Ernst [Reprint Author]
CORPORATE SOURCE: Biozentrum Klein Flottbek, Univ Hamburg, D-22609, Hamburg, Germany
eheinz@botanik.uni-hamburg.de
SOURCE: Journal of Lipid Research, (October 2004) Vol. 45, No. 10, pp. 1899-1909. print.
CODEN: JLPRAW. ISSN: 0022-2275.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Dec 2004
Last Updated on STN: 22 Dec 2004

AB In algae, the biosynthesis of docosahexaenoic acid (22:6omega3; DHA) proceeds via the elongation of eicosapentaenoic acid (20:5omega3; EPA) to 22:5omega3, which is required as a substrate for the final DELTA4 desaturation. To isolate the elongase specific for this step, we searched expressed sequence tag and genomic databases from the algae Ostreococcus tauri and Thalassiosira pseudonana, from the fish Oncorhynchus mykiss, from the frog Xenopus laevis, and from the sea squirt Ciona intestinalis using as a query the elongase sequence PpPSE1 from the moss **Physcomitrella patens**. The open reading frames of the identified elongase candidates were expressed in yeast for functional characterization. By this, we identified two types of elongases from O. tauri and T. pseudonana: one specific for the elongation of (DELTA6-)C18-PUFAs and one specific for (DELTA5-)C20-PUFAs, showing highest activity with EPA. The clones isolated from O. mykiss, X. laevis, and C. intestinalis accepted both C18- and C20-PUFAs. By coexpression of the DELTA6- and DELTA5-elongases from T. pseudonana and O. tauri, respectively, with the DELTA5- and DELTA4-desaturases from two other algae we successfully implemented DHA synthesis in stearidonic acid-fed yeast. This may be considered an encouraging first step in future efforts to implement this biosynthetic sequence into transgenic oilseed crops.

L15 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002424542 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12180987
TITLE: Cloning and functional characterization of Phaeodactylum tricornutum front-end desaturases involved in eicosapentaenoic acid biosynthesis.
AUTHOR: Domergue Frederic; Lerchl Jens; Zahringer Ulrich; Heinz Ernst
CORPORATE SOURCE: Institut fur Allgemeine Botanik, Universitat Hamburg, Hamburg, Germany.. fredDo@botanik.uni-hamburg.de
SOURCE: European journal of biochemistry / FEBS, (2002 Aug) 269 (16) 4105-13.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF503284; GENBANK-AY082392; GENBANK-AY082393
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 20020816
Last Updated on STN: 20021011

Entered Medline: 20021010

AB Phaeodactylum tricornutum is an unicellular silica-less diatom in which eicosapentaenoic acid accumulates up to 30% of the total fatty acids. This marine diatom was used for cloning genes encoding fatty acid desaturases involved in eicosapentaenoic acid biosynthesis. Using a combination of PCR, mass sequencing and library screening, the coding sequences of two desaturases were identified. Both protein sequences contained a cytochrome b5 domain fused to the N-terminus and the three histidine clusters common to all front-end fatty acid desaturases. The full length clones were expressed in Saccharomyces cerevisiae and characterized as Delta5- and Delta6-fatty acid desaturases. The substrate specificity of each enzyme was determined and confirmed their involvement in eicosapentaenoic acid biosynthesis. Using both desaturases in combination with the Delta6-specific elongase from **Physcomitrella patens**, the biosynthetic pathways of arachidonic and eicosapentaenoic acid were reconstituted in yeast. These reconstitutions indicated that these two desaturases functioned in the omega3- and omega6-pathways, in good agreement with both routes coexisting in Phaeodactylum tricornutum. Interestingly, when the substrate selectivity of each enzyme was determined, both desaturases converted the omega3- and omega6-fatty acids with similar efficiencies, indicating that none of them was specific for either the omega3- or the omega6-pathway. To our knowledge, this is the first report describing the isolation and biochemical characterization of fatty acid desaturases from diatoms.

L15 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 3

ACCESSION NUMBER: 2000395079 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10848999
TITLE: A bifunctional delta-fatty acyl acetylenase/**desaturase** from the moss Ceratodon purpureus. A new member of the cytochrome b5 superfamily.
AUTHOR: Sperling P; Lee M; Girke T; Zahringer U; Stymne S; Heinz E
CORPORATE SOURCE: Institut fur Allgemeine Botanik, Universitat Hamburg, Germany.
SOURCE: European journal of biochemistry / FEBS, (2000 Jun) 267 (12) 3801-11.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ250734; GENBANK-AJ250735
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000817

AB Many plant genes have been cloned that encode regioselective desaturases catalyzing the formation of cis-unsaturated fatty acids. However, very few genes have been cloned that encode enzymes catalyzing the formation of the functional groups found in unusual fatty acids (e.g. hydroxy, epoxy or acetylenic fatty acids). Here, we describe the characterization of an acetylenase from the moss Ceratodon purpureus with a regioselectivity differing from the previously described Delta12-acetylenase. The gene encoding this protein, together with a Delta6-**desaturase**, was cloned by a PCR-based approach with primers derived from conserved regions in Delta5-, Delta6-fatty-acid desaturases and Delta8-sphingolipid desaturases. The proteins that are encoded by the two cloned cDNAs are likely to consist of a N-terminal extension of unknown function, a cytochrome b5-domain, and a C-terminal domain that is similar to acyl lipid desaturases with characteristic histidine boxes. The proteins were highly homologous in sequence to the Delta6-**desaturase** from the moss **Physcomitrella patens**. When these two cDNAs were expressed in Saccharomyces cerevisiae, both transgenic yeast cultures desaturated Delta9-unsaturated C16- and C18-fatty acids by inserting an additional Delta6cis-double bond. One of these transgenic yeast clones was also able to introduce a Delta6-triple bond into gamma-linolenic and stearidonic acid. This resulted in the formation of 9,12,15-(Z,Z,Z)-octadecatrien-6-ynoic acid, the main fatty acid found in C. pupureus.

These results demonstrate that the Delta6-acetylenase from C. pupureus is a bifunctional enzyme, which can introduce a Delta6cis-double bond into 9,12,(15)-C18-polyenoic acids as well as converting a Delta6cis-double bond to a Delta6-triple bond.

L15 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 1998416756 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9744093
TITLE: Identification of a novel delta 6-acyl-group **desaturase** by targeted gene disruption in **Physcomitrella patens**.
AUTHOR: Girke T; Schmidt H; Zahringer U; Reski R; Heinz E
CORPORATE SOURCE: Universitat Hamburg, Institut fur Allgemeine Botanik, Germany.
SOURCE: Plant journal : for cell and molecular biology, (1998 Jul) 15 (1) 39-48.
Journal code: 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ222980; GENBANK-AJ222981
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981021
Last Updated on STN: 19981021
Entered Medline: 19981015

AB The moss **Physcomitrella patens** contains high levels of arachidonic acid. For its synthesis from linoleic acid by desaturation and elongation, novel delta 5- and delta 6-desaturases are required. To isolate one of these, PCR-based cloning was used, and resulted in the isolation of a full-length cDNA coding for a putatively new **desaturase**. The deduced amino acid sequence has three domains: a N-terminal segment of about 100 amino acids, with no similarity to any sequence in the data banks, followed by a cytochrome b5-related region and a C-terminal sequence with low similarity (27% identify) to acyl-lipid desaturases. To elucidate the function of this protein, we disrupted its gene by transforming P. **patens** with the corresponding linear genomic sequence, into which a positive selection marker had been inserted. The molecular analysis of five transformed lines showed that the selection cartridge had been inserted into the corresponding genomic locus of all five lines. The gene disruption resulted in a dramatic alteration of the fatty acid pattern in the knockout plants. The large increase in linoleic acid and the concomitant disappearance of gamma-linolenic and arachidonic acid in all knockout lines suggested that the new cDNA coded for a delta 6-**desaturase**. This was confirmed by expression of the cDNA in yeast and analysis of the resultant fatty acids by GC-MS. Only the transformed yeast cells were able to introduce a further double bond into the delta 6-position of unsaturated fatty acids. To our knowledge, this is the first report of a successful gene disruption in a multicellular plant resulting in a specific biochemical phenotype.

=>

L19 ANSWER 1 OF 4 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2005329554 EMBASE
TITLE: In vivo characterization of the first acyl-CoA Δ(6)- **desaturase** from a member of the plant kingdom, the microalga Ostreococcus tauri.
AUTHOR: Domergue F.; Abbadi A.; Zahringer U.; Moreau H.; Heinz E.
CORPORATE SOURCE: F. Domergue, Biozentrum Klein Flottbek, Universitat Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany. fredomergue@voila.fr
SOURCE: Biochemical Journal, (15 Jul 2005) Vol. 389, No. 2, pp. 483-490.
Refs: 32
ISSN: 0264-6021 CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20050811
Last Updated on STN: 20050811

AB Genomic DNA of Ostreococcus tauri, a fully sequenced marine unicellular alga from the phytoplankton, was used to amplify a gene coding for a typical front-end desaturase involved in polyunsaturated fatty acid biosynthesis. Heterologous expression in Saccharomyces cerevisiae revealed very high desaturation activity with Δ(6)-regioselectivity. Short-time kinetic experiments showed that the desaturase product was detected in the acyl-CoA pool 5 min after addition of the exogenous substrate to the yeast medium and long before its appearance in the total fatty acids. When this desaturase was co-expressed with the acyl-CoA Δ(6)-elongase from **Physcomitrella patens** and the lipid-linked Δ(5)-desaturase from Phaeodactylum tricornutum, high proportions of arachidonic or eicosapentaenoic acid were obtained, because nearly all of the Δ(6)-desaturated products were elongated. Furthermore, the product/educt ratios calculated in each glycerolipid for the Δ(**6**)-**desaturase** or for the acyl-CoA Δ(6)-elongase were in about the same range, whereas this ratio showed a very uneven profile in the case of the lipid-linked Δ(5)-desaturase. Finally, a sequence-based comparison of all the functionally characterized Δ(6)-desaturases showed that this enzyme was not related to any previously described sequence. Altogether, our data suggest that this desaturase from O. tauri is an acyl-CoA Δ(**6**)-**desaturase**, the first one cloned from a photosynthetically active organism. .COPYRGT. 2005 Biochemical Society.

L19 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:219501 BIOSIS
DOCUMENT NUMBER: PREV200510002914
TITLE: Metabolic engineering of new fatty acids in plants.
AUTHOR(S): Singh, Surinder P.; Zhou, Xue-Rong; Liu, Qing; Stymne, Sten; Green, Allan G. [Reprint Author]
CORPORATE SOURCE: CSIRO Plant Ind, POB 1600, Canberra, ACT 2601, Australia Allan.Green@csiro.au
SOURCE: Current Opinion in Plant Biology, (APR 2005) Vol. 8, No. 2, pp. 197-203.
ISSN: 1369-5266.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Jun 2005
Last Updated on STN: 10 Jun 2005

AB Metabolic engineering of plants to express high levels of new fatty acids that are of nutritional and industrial importance has proven to be highly challenging. Significant advances have been made recently, however, particularly in the development of the first plant oils to contain long-chain polyunsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. Methods of increasing the

accumulation of Delta 12-modified fatty acids synthesized by transgenically expressed FAD2-like enzymes have also been investigated. Biochemical analyses of plants that express these introduced fatty-acid metabolic pathways have highlighted the central importance of ensuring the removal of novel fatty acids from their site of synthesis on phosphatidylcholine to enable their further modification, exclusion from membrane lipids and accumulation in seed triacylglycerols.

L19 ANSWER 3 OF 4 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 1

ACCESSION NUMBER: 2000230423 EMBASE
TITLE: A bifunctional Δ6-fatty acyl acetylenase/desaturase from the moss Ceratodon purpureus: A new member of the cytochrome b5 superfamily.
AUTHOR: Sperling P.; Lee M.; Girke T.; Zahringer U.; Styrune S.; Heinz E.
CORPORATE SOURCE: E. Heinz, Institut fur Allgemeine Botanik, Ohnhorststr. 18, D-22609 Hamburg, Germany. eheinz@botanik.uni-hamburg.de
SOURCE: European Journal of Biochemistry, (2000) Vol. 267, No. 12, pp. 3801-3811.
Refs: 36
ISSN: 0014-2956 CODEN: EJBCAI
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20000720
Last Updated on STN: 20000720

AB Many plant genes have been cloned that encode regioselective desaturases catalyzing the formation of cis-unsaturated fatty acids. However, very few genes have been cloned that encode enzymes catalyzing the formation of the functional groups found in unusual fatty acids (e.g. hydroxy, epoxy or acetylenic fatty acids). Here, we describe the characterization of an acetylenase from the moss Ceratodon purpureus with a regioselectivity differing from the previously described Δ12-acetylenase. The gene encoding this protein, together with a Δ **6-desaturase**, was cloned by a PCR-based approach with primers derived from conserved regions in Δ5-, Δ6-fatty- acid desaturases and Δ8-sphingolipid desaturases. The proteins that are encoded by the two cloned cDNAs are likely to consist of a N-terminal extension of unknown function, a cytochrome b5-domain, and a C-terminal domain that is similar to acyl lipid desaturases with characteristic histidine boxes. The proteins were highly homologous in sequence to the Δ **6- desaturase** from the moss **Physcomitrella patens**. When these two cDNAs were expressed in Saccharomyces cerevisiae, both transgenic yeast cultures desaturated Δ9-unsaturated C16- and C18-fatty acids by inserting an additional Δ6-cis-double bond. One of these transgenic yeast clones was also able to introduce a Δ6-triple bond into γ-linolenic and stearidonic acid. This resulted in the formation of 9,12,15-(Z,Z,Z)-octadecatrien-6-ynoic acid, the main fatty acid found in C. pupureus. These results demonstrate that the Δ6-acetylenase from C. pupureus is a bifunctional enzyme, which can introduce a Δ6-cis-double bond into 9,12,(15)-C18-polyenoic acids as well as converting a Δ6-cis-double bond to a Δ6-triple bond.

L19 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 1998416756 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9744093
TITLE: Identification of a novel delta **6**-acyl-group **desaturase** by targeted gene disruption in **Physcomitrella patens**.
AUTHOR: Girke T; Schmidt H; Zahringer U; Reski R; Heinz E
CORPORATE SOURCE: Universitat Hamburg, Institut fur Allgemeine Botanik, Germany.
SOURCE: Plant journal : for cell and molecular biology, (1998 Jul) 15 (1) 39-48.

Journal code: 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ222980; GENBANK-AJ222981
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981021
Last Updated on STN: 19981021
Entered Medline: 19981015

AB The moss **Physcomitrella patens** contains high levels of arachidonic acid. For its synthesis from linoleic acid by desaturation and elongation, novel delta 5- and delta 6-desaturases are required. To isolate one of these, PCR-based cloning was used, and resulted in the isolation of a full-length cDNA coding for a putatively new desaturase. The deduced amino acid sequence has three domains: a N-terminal segment of about 100 amino acids, with no similarity to any sequence in the data banks, followed by a cytochrome b5-related region and a C-terminal sequence with low similarity (27% identify) to acyl-lipid desaturases. To elucidate the function of this protein, we disrupted its gene by transforming P. **patens** with the corresponding linear genomic sequence, into which a positive selection marker had been inserted. The molecular analysis of five transformed lines showed that the selection cartridge had been inserted into the corresponding genomic locus of all five lines. The gene disruption resulted in a dramatic alteration of the fatty acid pattern in the knockout plants. The large increase in linoleic acid and the concomitant disappearance of gamma-linolenic and arachidonic acid in all knockout lines suggested that the new cDNA coded for a delta **6-desaturase**. This was confirmed by expression of the cDNA in yeast and analysis of the resultant fatty acids by GC-MS. Only the transformed yeast cells were able to introduce a further double bond into the delta 6-position of unsaturated fatty acids. To our knowledge,

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